nature portfolio

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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Custom scripts used in this study for sgRNA abundance analysis, data quality assessment, and differential gene expression analysis are publicly available on Zenodo at https://doi.org/10.5281/zenodo.15179309.
Data analysis	CRISPRi-seq sequencing reads were analyzed using 2FAST2Q(v2.5.3). Differential abundance analysis was done with Rstudio using DESeq2 (v1.40.2). Flow cytometry analysis was performed with FlowJo software (version 10). GraphPad Prism (version 8.4.3) and R (version 4.3.1).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All raw sequencing data supporting this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession PRJNA1063516 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1063516/).

The dataset spans SRA accessions SRR27547817-SRR27547879 and SRR32432725-SRR32432777, with corresponding BioSample accession (SAMN39398487-SAMN39398491, SAMN46956771-SAMN46956772) available through the NCBI BioSample database.
Other data generated in this study are provided in the Supplementary Information/Source Data file. Source Data are provided with this paper.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Not applicable. This study did not involve human participants, human data, or human tissue.
Reporting on race, ethnicity, or other socially relevant groupings	Not applicable. This study did not involve human participants, human data, or human tissue.
Population characteristics	Not applicable. This study did not involve human participants, human data, or human tissue.
Recruitment	Not applicable. This study did not involve human participants, human data, or human tissue.
Ethics oversight	Not applicable. This study did not involve human participants, human data, or human tissue.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical method was used to predetermine sample size. The sample sizes for in vitro bacterial experiments and in vivo mouse studies were based on commonly accepted standards in the field and were sufficient to observe reproducible effects. For mouse infection experiments, 6 animals per group were used, which was based on prior experience and is consistent with published studies using similar infection models to achieve adequate statistical power while minimizing animal use.
Data exclusions	All data points and samples that were successfully collected and passed quality control were included in the analysis. No animals or samples were excluded.
Replication	All in vitro experiments were performed with at least three independent biological replicates unless otherwise specified. Animal experiments were conducted using independent groups of mice, with 6 animals per group.
Randomization	For in vitro bacterial experiments, randomization was not applicable as experimental samples consisted of bacterial cultures processed according to predefined protocols without allocation into groups. For animal experiments, mice were randomly allocated into experimental groups at the time of infection and treatment to minimize bias. No specific covariates required control.
Blinding	No blinding was performed during in vitro experiments, as the nature of the assays (e.g., bacterial growth measurements) made blinding impractical and unnecessary. In animal experiments, investigators were aware of group allocation during the experiments. However, quantitative outcomes, including bacterial burden in lungs and survival, were objectively measured, reducing the risk of assessment bias.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Female BALB/c mice (18-22 g, 6-8 weeks old) were purchased from Guangdong Medical Laboratory Animal Center and maintained under specific pathogen-free (SPF) conditions. The mice were housed under SPF conditions with a 12-hour light/dark cycle, ambient temperature of 22 ± 2 °C, and 40–60% relative humidity.
Wild animals	No wild animals were used in this study.
Reporting on sex	This study exclusively used female mice. Female mice were selected to minimize variability caused by potential sex-related behavioral differences (e.g., aggression) and to maintain consistency across experimental groups. Sex-specific effects were not assessed, as no comparative phenotypic data from male mice were collected in this study. Therefore, conclusions cannot be generalized to both sexes, and further investigations would be required to evaluate potential sex-based differences.
Field-collected samples	Not applicable to this study as no field-collected samples was used.
Ethics oversight	The study protocol of animal experiments was approved by the Institutional Animal Care and Use Committee of Shenzhen University Medical School (Approval Number: IACUC-202400111).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	not applicable
Novel plant genotypes	not applicable
Authentication	not applicable

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Bacterial cell cultures (OD600 = 0.5–0.7) were diluted to OD600 = 0.01 in LB broth and incubated at 37°C for 4 hours. Carboxy-H2DCFDA (10 µM) or PeroxyOrange-1 (5 µM) was then added, followed by a 2-hour dark incubation at 37°C, shaking at 250 rpm. Unstained controls assessed background fluorescence.
Instrument	NovoCyte Advanteon Flow Cytometer (Agilent) and BD FACSAria II Flow Cytometer (BD Biosciences).

Software	FlowJo software (version 10).
Cell population abundance	Flow cytometry acquisition captured 100,000 ungated events per sample using standardized parameters.
Gating strategy	Single live cells were gated (G1) using FSC-A/SSC-A parameters, followed by FSC-H/FSC-A doublet exclusion. Fluorescence thresholds were defined as fluorescence intensity exceeding 2-fold over unstained controls, with background signal quantified from non-stained parallel samples.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.